

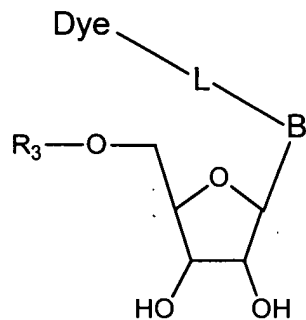
CLAIM LISTING:

This listing of claims will replace all prior versions and listings of claims in the application::

Claims 1-100 (cancelled)

101 (previously presented). A method for determining a polynucleotide sequence, comprising

- (i) annealing at least one primer to a template polynucleotide;
- (ii) extending said at least one primer in the presence of a mixture of unlabeled dNTPs and at least one dye-labeled ribonucleotide having the formula:



wherein B is a nucleobase; L is a linker; R₃ is triphosphate, α -thiotriphosphate, or a salt thereof, and Dye is a reporter group;

so that primer extension products that contain at least one dye-labeled ribonucleotide are formed;

(iii) cleaving one or more primer extension products to form a plurality of labeled fragments;

(iv) separating the extension products by size; and

(v) detecting the fragments to determine the polynucleotide sequence.

102 (original). The method according to claim 101, wherein the dye-labeled ribonucleotides are rATP-PA-6R6G, rCTP-PA-Rox, rUTP-PA-Tamra and rGTP-EO-R110.

103 (original). The method according to claim 101, wherein one primer is biotinylated.

104 (original). The method according to claim 101, wherein at least one primer is a hybridization based pull-out primer.

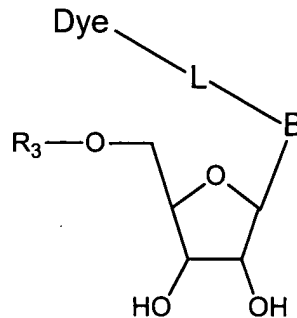
105 (original). The method according to claim 101, wherein the DNA polymerase is a thermostable DNA polymerase.

106 (original). The method according to claim 105, wherein the thermostable DNA polymerase is a modified thermostable DNA polymerase having increased efficiency for the incorporation of ribonucleotides.

107 (currently amended). The method according to claim 101, wherein ~~the means for hydrolyzing the extension products~~ said one or more primer extension products are cleaved at each occurrence of a ribonucleotide ~~is~~ by alkali treatment, heat treatment, or a ribonuclease.

108 (previously presented). A method for detecting mutations in a polynucleotide, comprising

- annealing two primers to a template polynucleotide;
- extending the two primers in the presence of a mixture of unlabeled dNTPs and at least one dye-labeled ribonucleotide having the formula:



- wherein B is a nucleobase; L is a linker; R₃ is triphosphate, α -thiotriphosphate, or a salt thereof, and Dye is a reporter group;
- so that primer extension products that contain at least one dye-labeled ribonucleotide are formed;
- cleaving one or more primer extension products to form a plurality of labeled fragments;
 - separating the fragments by size; and
 - detecting the fragments to detect the mutations.

109 (previously presented). The method according to claim 108, wherein the fragments that contain primers are separated from other fragments before the fragments that contain primers are separated by size.

110 (original). The method according to claim 108, wherein the mutation is a single nucleotide polymorphism.

111 (previously presented). The method according to claim 108, wherein the polynucleotide is genomic DNA.

112 (original). The method according to claim 108, wherein at least one primer is biotinylated.

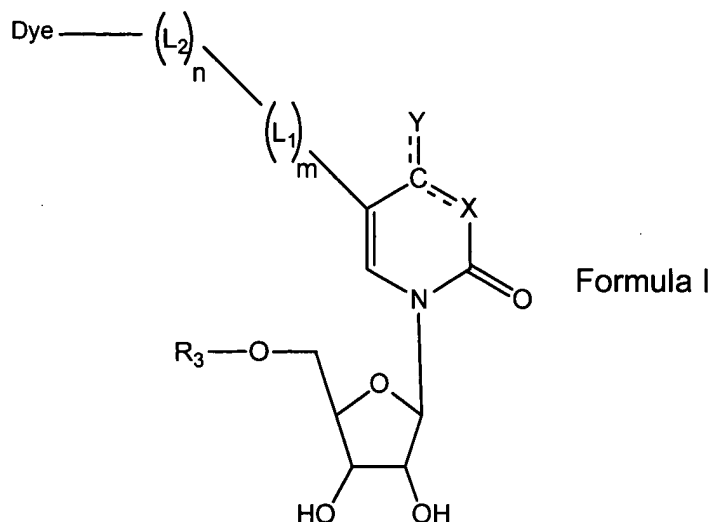
113 (original). The method according to claim 108, wherein at least one primer is a hybridization based primer.

114 (original). The method according to claim 108, wherein one primer comprises a modified base preventing primer extension in the 5' direction.

Claims 115-123 (cancelled)

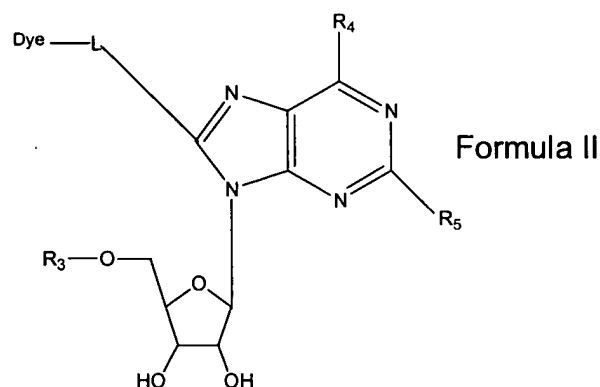
124 (previously presented). The method according to claim 101, wherein said at least one dye-labeled ribonucleotide is:

(1) a compound of formula I:



- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L₁ is a linker;
- wherein L₂ is a benzylamine linker or a phosphate linker;
- wherein n = 0-4, m = 0-4, and m + n is at least 1; and;
- wherein the dye is any reporter group;

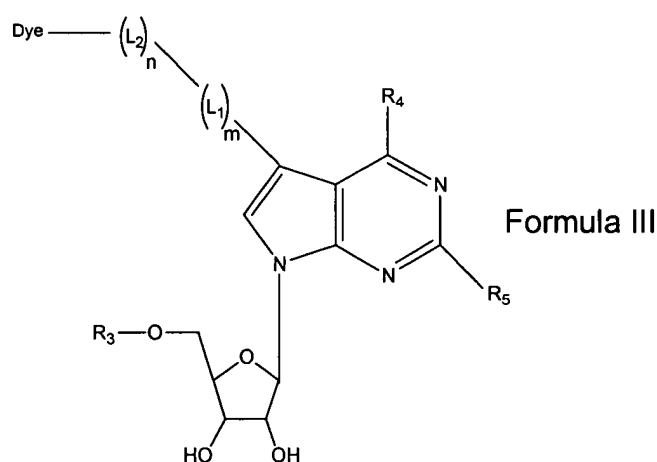
(2) a compound of formula II:



- wherein L is a linker;
- wherein R₄ is either NH₂, OH, or O, and B is either NH₂, OH, or H;

- wherein R_3 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- and
- wherein the dye is any reporter group;

(3) a compound of formula III:

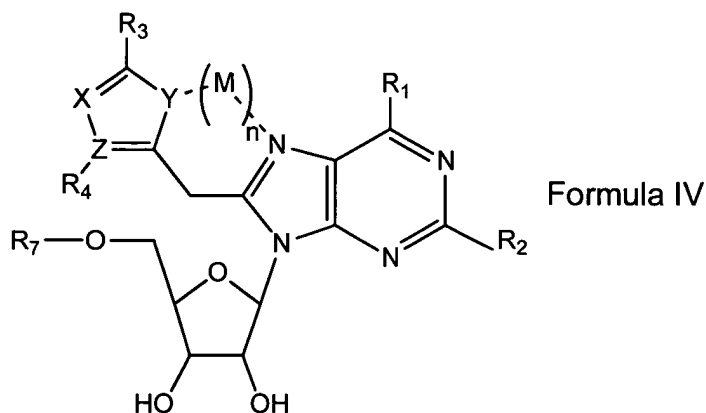


- wherein L_1 is a linker;
- wherein L_2 is a benzylamine linker or a phosphate linker;
- wherein $n = 0-4$, $m = 0-4$, and $m + n$ is at least 1;
- wherein R_4 is either NH_2 , OH , or O , and R_5 is either NH_2 , OH , or H ;
- wherein R_3 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- and
- wherein the dye is any reporter group;

(4) a compound of formula IV:

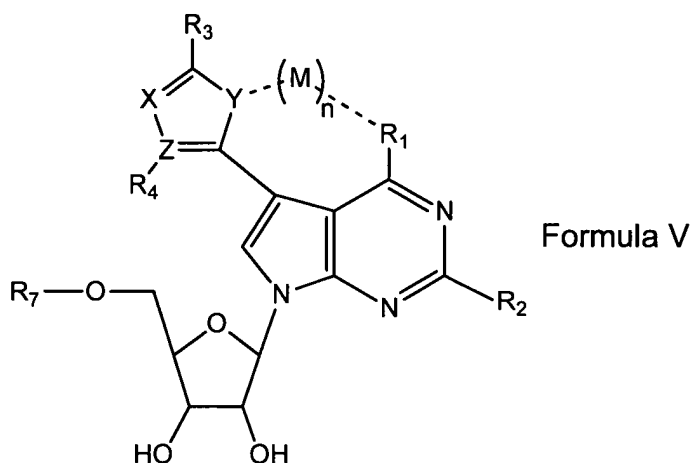
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- wherein R_1 , R_2 , and R_4 are independently H, O, OR, S, SR, NR_2 or CR_2 ;
- wherein R_3 is SR, NR_2 , OR, or CR_2 and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein R_7 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is H_2O or any metal;

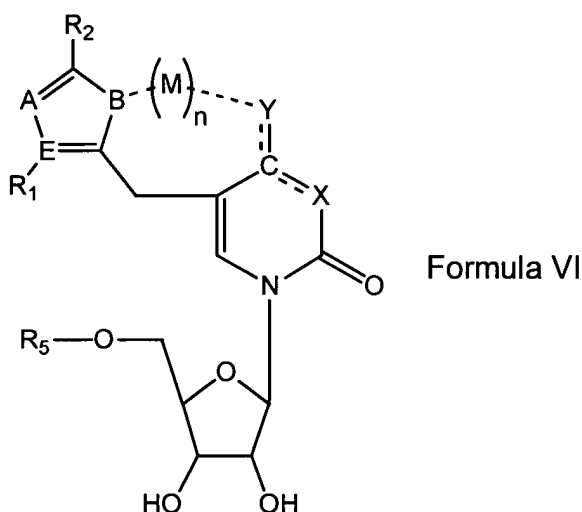
(5) a compound of formula V:



- wherein R_1 , R_2 , and R_4 are independently H, O, OR, S, SR, NR_2 or CR_2 ;
- wherein R_3 is SR, NR_2 , OR, or CR_2 and comprises a reporter group;

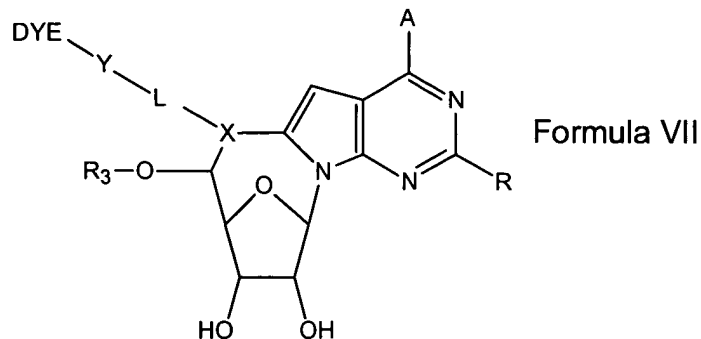
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein R₇ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is H₂O or any metal;

(6) a compound of formula VI:



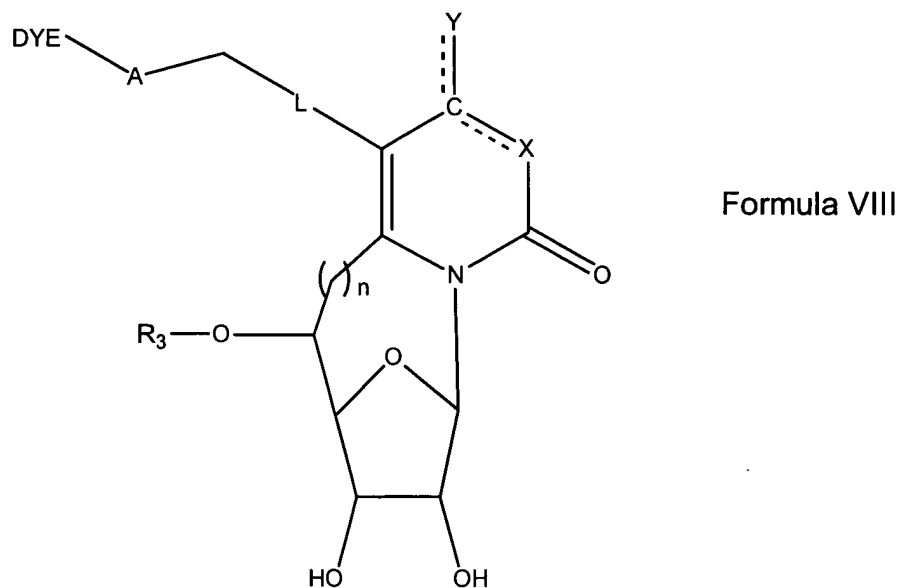
- wherein R₁ is H, O, OR, S, SR, NR₂, or CR₂,
- wherein R₂ is SR, NR₂, OR, or CR₂ and comprises a reporter group;
- wherein R is hydrogen, alkyl, alkynyl, aryl, or an amino acid;
- wherein R₅ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein A, B, and E are independently C, N, O, S, P, or Se;
- wherein n is 0 or 1; and
- wherein M is H₂O or any metal;

(7) a compound of formula VII:



- wherein A is NH₂, OH, or O;
- wherein R is H, O, NR'₂, S, CR'₂, or halide;
- wherein R' is hydrogen or alkyl;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L is alkyl;
- wherein X is CR or N and Y is O, S, or NH; and
- wherein the dye is any reporter group;

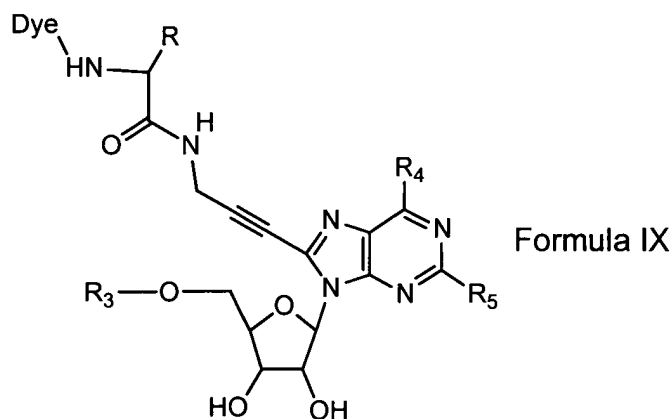
(8) a compound of formula VIII:



- wherein X is N, NH, or C;

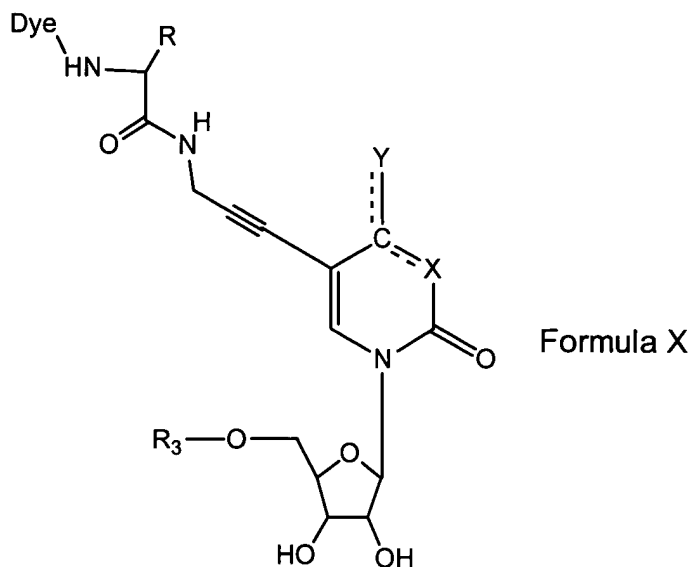
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein A is O, S, or NH;
- wherein L is alkyl or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO₂R', SO₃, or NR'₂;
- wherein R' is hydrogen or alkyl;
- wherein n is 1 to 10; and
- wherein the dye is any reporter group;

(9) a compound of formula IX:



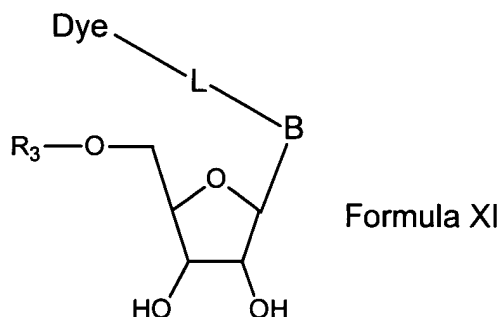
- wherein R₄ is NH₂, OH, or O and R₅ is NH₂, OH, or H, provided that if A is NH₂, B is H and if A is O, B is NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein the dye is any reporter group; and
- wherein R is a side chain for mobility tuning;

(10) a compound of formula X:



- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein Dye is any reporter group, and
- wherein R is a side chain for mobility tuning;

(11) a compound of formula XI:



- wherein B is a nucleobase selected from uracil, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanine;
- wherein R₃ is triphosphate or a salt thereof;

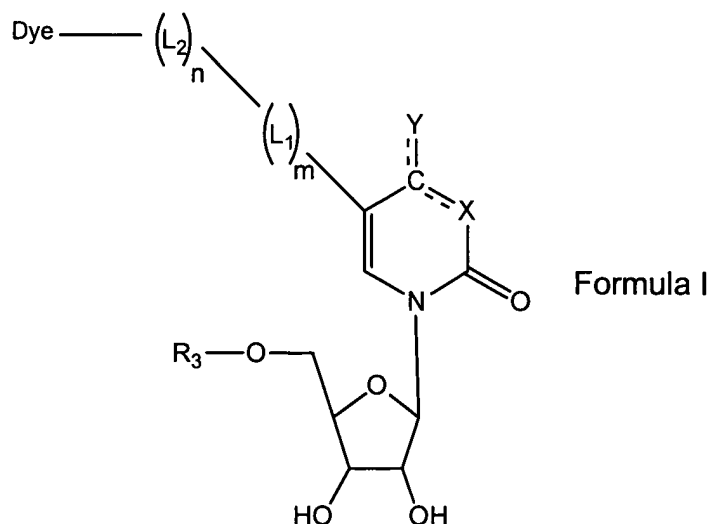
- wherein L is a linker selected from propargyl-ethyl-oxide-amino and propargylamino wherein the linker is attached to the 8-C of a adenine, 7-deazaadenine, guanine, or 7-deazaguanine nucleobase, the 7-C or 8-C of a 7-deazaadenine or 7-deazaguanine nucleobase, or the C-5 of a uracil or cytosine nucleobase; and
- wherein Dye is selected from a rhodamine dye and a fluorescein dye.

125 (previously presented). The method according to claim 101, wherein the reporter group is a rhodamine-type dye, a fluorescein-type dye, an energy transfer dye, or a cyanine-type dye.

126 (previously presented). The method according to claim 101, further comprising separating the fragments that contain at least one primer from other fragments.

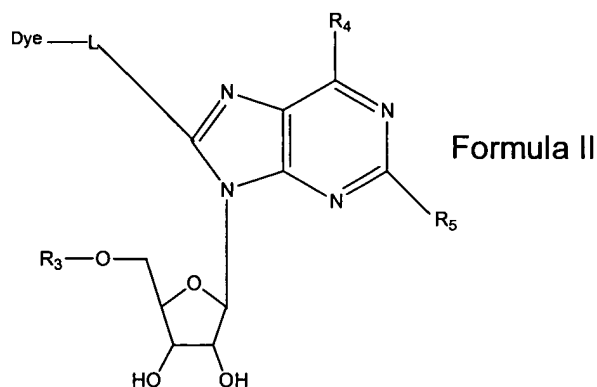
127 (previously presented). The method according to claim 108, wherein said at least one dye-labeled ribonucleotide is:

- (1) a compound of formula I:



- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L₁ is a linker;
- wherein L₂ is a benzylamine linker or a phosphate linker;
- wherein n = 0-4, m = 0-4, and m + n is at least 1; and;
- wherein the dye is any reporter group;

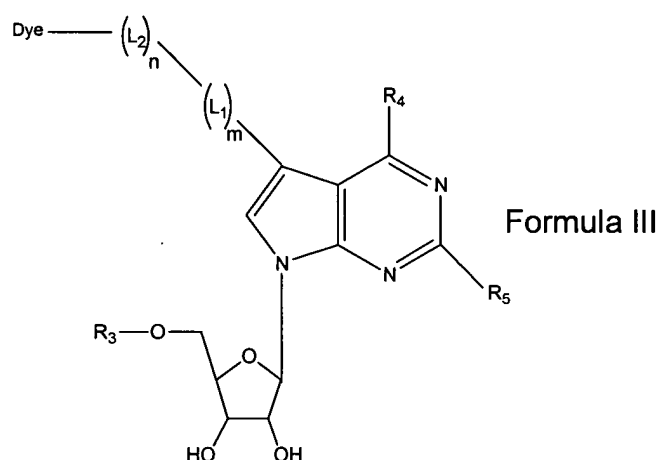
(2) a compound of formula II:



- wherein L is a linker;
- wherein R₄ is either NH₂, OH, or O, and B is either NH₂, OH, or H;

- wherein R_3 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- and
- wherein the dye is any reporter group;

(3) a compound of formula III:

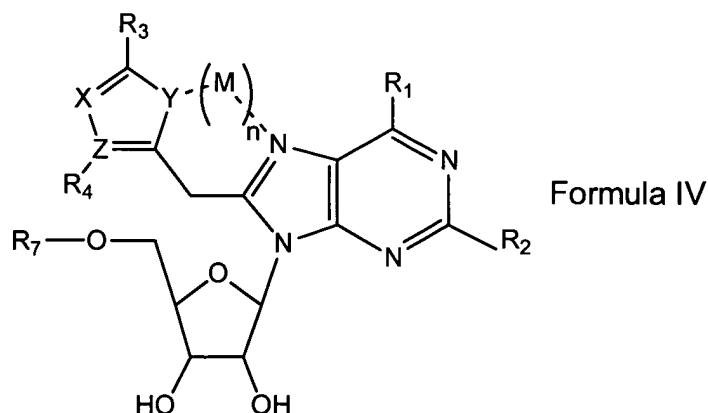


- wherein L_1 is a linker;
- wherein L_2 is a benzylamine linker or a phosphate linker;
- wherein $n = 0-4$, $m = 0-4$, and $m + n$ is at least 1;
- wherein R_4 is either NH_2 , OH , or O , and R_5 is either NH_2 , OH , or H ;
- wherein R_3 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- and
- wherein the dye is any reporter group;

(4) a compound of formula IV:

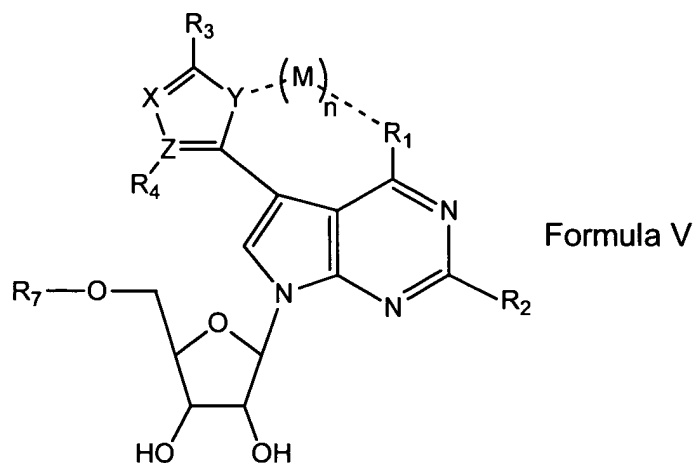
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- wherein R_1 , R_2 , and R_4 are independently H, O, OR, S, SR, NR_2 or CR_2 ;
- wherein R_3 is SR, NR_2 , OR, or CR_2 and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein R_7 is either triphosphate, α -thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is H_2O or any metal;

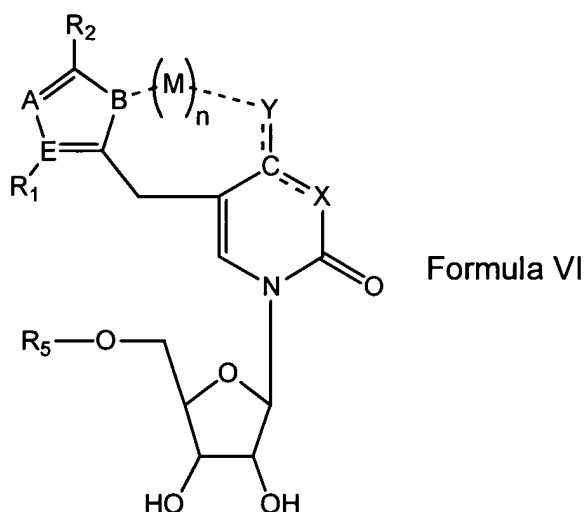
(5) a compound of formula V:



- wherein R_1 , R_2 , and R_4 are independently H, O, OR, S, SR, NR_2 or CR_2 ;
- wherein R_3 is SR, NR_2 , OR, or CR_2 and comprises a reporter group;

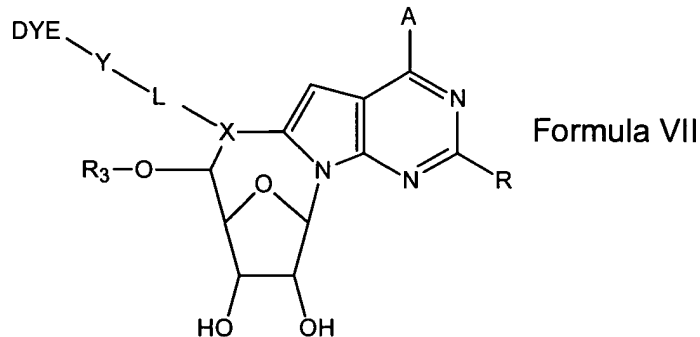
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein R₇ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is H₂O or any metal;

(6) a compound of formula VI:



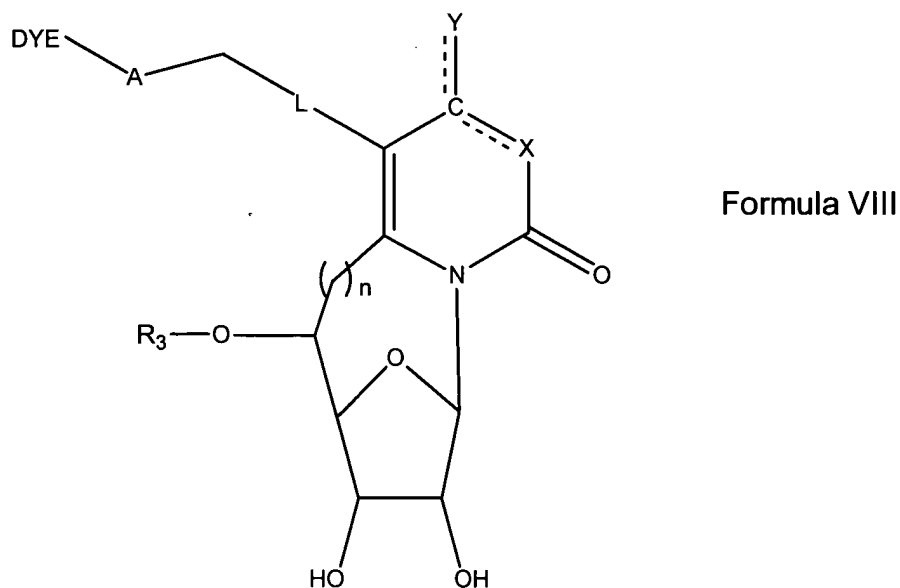
- wherein R₁ is H, O, OR, S, SR, NR₂, or CR₂,
- wherein R₂ is SR, NR₂, OR, or CR₂ and comprises a reporter group;
- wherein R is hydrogen, alkyl, alkynyl, aryl, or an amino acid;
- wherein R₅ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein A, B, and E are independently C, N, O, S, P, or Se;
- wherein n is 0 or 1; and
- wherein M is H₂O or any metal;

(7) a compound of formula VII:



- wherein A is NH₂, OH, or O;
- wherein R is H, O, NR'₂, S, CR'₂, or halide;
- wherein R' is hydrogen or alkyl;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L is alkyl;
- wherein X is CR or N and Y is O, S, or NH; and
- wherein the dye is any reporter group;

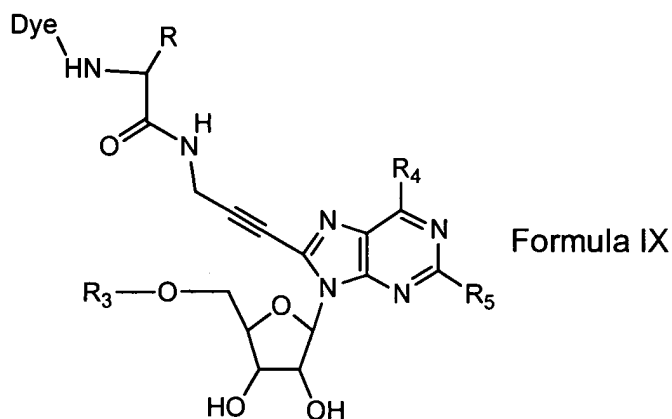
(8) a compound of formula VIII:



- wherein X is N, NH, or C;

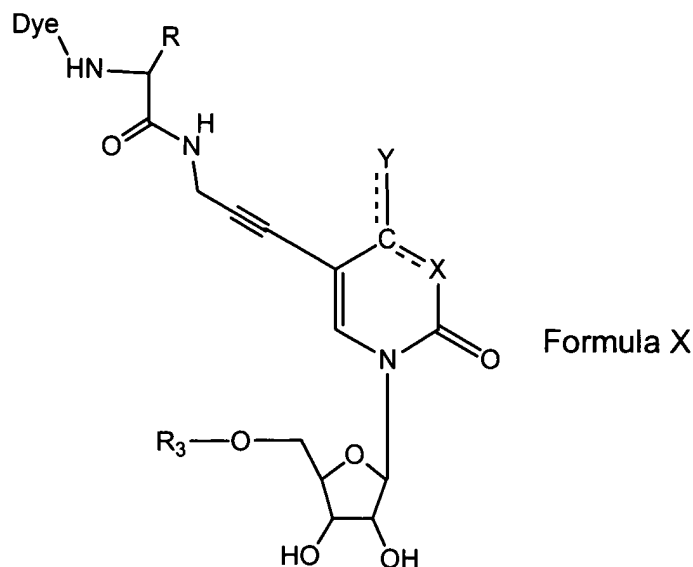
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein A is O, S, or NH;
- wherein L is alkyl or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO₂R', SO₃, or NR'₂;
- wherein R' is hydrogen or alkyl;
- wherein n is 1 to 10; and
- wherein the dye is any reporter group;

(9) a compound of formula IX:



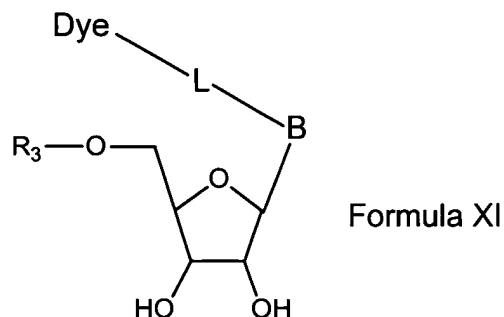
- wherein R₄ is NH₂, OH, or O and R₅ is NH₂, OH, or H, provided that if A is NH₂, B is H and if A is O, B is NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein the dye is any reporter group; and
- wherein R is a side chain for mobility tuning;

(10) a compound of formula X:



- wherein X is N, NH, or C;
- wherein Y is O or NH₂;
- wherein R₃ is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein Dye is any reporter group, and
- wherein R is a side chain for mobility tuning;

(11) a compound of formula XI:



- wherein B is a nucleobase selected from uracil, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanine;
- wherein R₃ is triphosphate or a salt thereof;

- wherein L is a linker selected from propargyl-ethyl-oxide-amino and propargylamino wherein the linker is attached to the 8-C of a adenine, 7-deazaadenine, guanine, or 7-deazaguanine nucleobase, the 7-C or 8-C of a 7-deazaadenine or 7-deazaguanine nucleobase, or the C-5 of a uracil or cytosine nucleobase; and
- wherein Dye is selected from a rhodamine dye and a fluorescein dye.

128 (previously presented). The method according to claim 108, wherein the reporter group is a rhodamine-type dye, a fluorescein-type dye, an energy transfer dye, or a cyanine-type dye.

129 (previously presented). The method according to claim 108, further comprising separating the fragments that contain at least one primer from other fragments.